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THE BACILLUS OF LEPROSY.

A MICROSCOPICAL STUDY OF ITS MORPHOLOGICAL CHARACTERISTICS.*

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The bacillus of leprosy, belonging to the class of pathogenic or disease-producing organisms, is met with as a fine, rod-shaped bacillus, rounded or slightly pointed at the extremities, and averaging about 5μ in length, by a little less than 1μ in diameter. Some individual bacilli may be observed with bright, oval spores, and many, though not all, present an active to-and-fro motion. In quite a number of specimens there is a beaded appearance, owing to the local centralization of protoplasmic masses. This shows out clearly in some of the bacilli in Fig. 1, accompanying this paper.

The bacilli are found most abundantly in the leprous nodules of the skin, in which they literally swarm, appearing in some portions almost as plentiful as the cells of the tissue itself. They are also very plentiful in the leprous lesions of the buccal, laryngeal, and other cavities of the body. Some bacilli are found in the internal organs, as the kidneys, testicles, liver, spleen, and lymphatic system; though, with probably the exception of the latter, they do not usually exist in such large numbers as in the skin. In the anæsthetic variety of the disease, or in the anæsthetic patches of cases where both varieties coexist in the same victim, the bacilli are found in considerable numbers in the interstices between the filaments of the various nerve tissues.

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* The figures accompanying this paper are reproductions of the author's camera-lucida drawings. Photographs of these were submitted with the paper at the Columbus meeting. Microscopical mounts and lantern slides also sent, were unfortunately not received through some failure in the mail.— SECRETARY.

There are several good methods of staining *Bacillus leprae*, but that which I have found to be the most satisfactory for the cover-glass preparations, is the acid solution of eosin-hæmatoxylin of Ehrlich. Sections also may be stained in this manner; but a method which I consider more satisfactory is that of Babes. It consists in first staining with a solution of rosaniline hydrochlorate in aniline-water. Then bleach with a solution of hydrochloric acid in water (1 part to 4), afterwards re-staining with methylene-blue.

While visiting the hospitals of Europe some years ago it was my good fortune to meet in the *Allgemeines Krankenhaus* in Vienna, a case of leprosy from which I obtained some serum and a small portion of a leprous tubercle of the skin. And recently my friend, Dr. Jno. B. Harper, who has been traveling around the world, has sent me from Honolulu in the Sandwich Islands a small portion of the liver and kidney of a woman dead with leprosy. From these two cases I have made a number of preparations, and surmising that they would interest you I present them to-day for your inspection, together with some photographs and lantern slides made from camera-lucida drawings. These preparations were obtained in two ways:

First, — From the leprous tubercles of the skin for cover-glass preparations, by the method of Mansen; which consists in compressing the tubercle with a pile clamp until most of the blood has been forced from the tissues, when, if pricked with a needle, a thin serous fluid will exude, which can be prepared on a cover-glass in the ordinary way. When stained as before mentioned it will be found to contain the bacilli in large numbers, if the tubercle has been one of true leprosy. Slide No. 1 was prepared in this way.

Second,—By sections cut from the leprous nodules of the skin, and from the lung and kidney—all of these organs being affected in the two cases from which I obtained specimens.

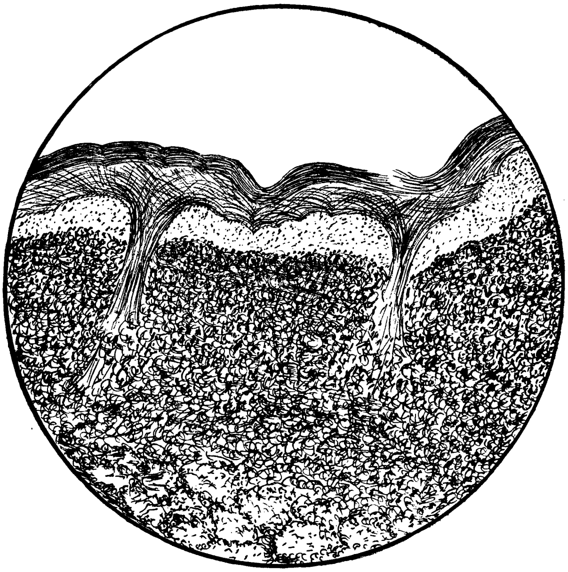

Fig. 1.

Fig. 1 represents a section vertically through a nodule of the skin; the case being one of tubercular leprosy. The rete Malpighii may be seen somewhat thickened and swollen by the infiltration with leucocytes and large, characteristic, lepra cells. These latter are undoubtedly wandering cells. which have been enlarged and distended by the growth within them, of the bacilli, as pointed out by Neisser. A large excess of pigment granules will also be noticed, which is due to the increased deposit in consequence of the morbid process going on in the tissues, producing in some cases such intense pigmentation, in spots, as to give rise to the term "macular leprosy."

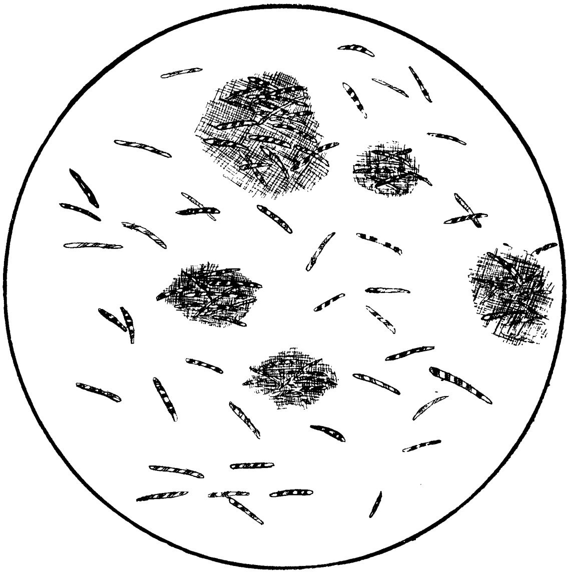

Fig. 2.

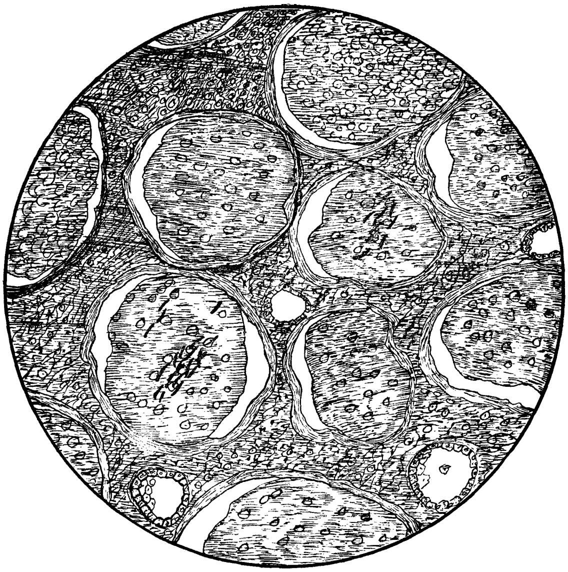

Fig. 3.

Fig. 2 represents a higher magnification of the same specimen. In it can be seen large numbers of the bacilli, occupying the large lepra cells of Virchow, while surrounding these are single bacilli and spores. The latter, while bright and glistening, and in large numbers as seen through the microscope, are not shown in the drawing. Some of the bacilli in the slide from which this was taken show minute, sparkling crystals of sulphur, which also do not appear. In all other respects this is a very accurate representation of what was to be seen.

Fig. 3 represents a section of the kidney, prepared from the specimen sent me by Dr. Harper. Two small clusters of bacilli may be seen in the center of two glomeruli. Under a $\frac{1}{16}$ in. objective these organisms presented the typical appearance of *Bacillus leprae*, which is easily recognized. Although, in the absence of the clinical history of the case as corroboration, it would be impossible to assert from the appearance alone the certain identity as distinguished from *Bacillus tuberculosis;*

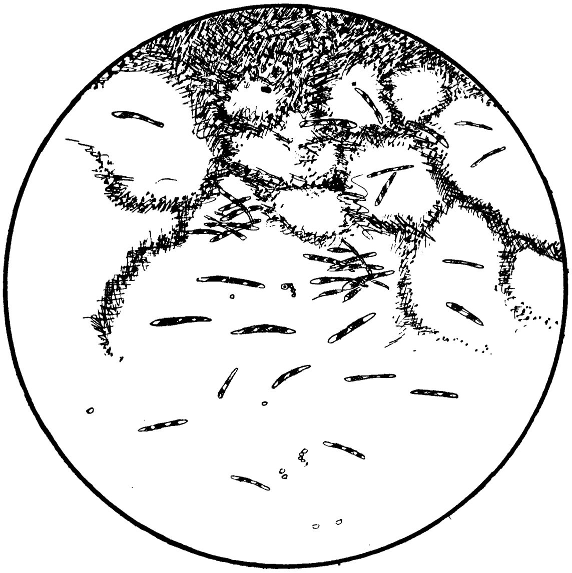

Fig. 4.

yet, when taken in connection with other characteristics as given hereafter, it is not a very difficult matter to make the distinction. And with the present rapid rate of progress in microscopical science it will undoubtedly not be long until all the many species of bacilli may be distinguished from each other, almost as easily as we now recognize the morphological difference between the various classes of micro-organisms.

Fig. 4 represents a field from a section of liver. Masses of bacilli may be seen, together with a number of leprosy cells. The liver structure itself is very much broken down, and infiltrated with inflammatory new formation. A few blood vessels are visible, the coats of which are also much thickened and infiltrated by the local morbid process.

Slide No. 1 is a cover-glass preparation of the serum from a leprous tubercle of the skin. It shows bacilli, some enclosed in the interior of the giant cells, others are floating free in the serum, interspersed with pus corpuscles and debris of all kinds.

Slide No. 2 is taken from an artificial culture, and, as will be seen, shows the change which has taken place in the form of the bacillus as the result of cultivation. The organism, instead of presenting short, fine rods, has grown into long, jointed filaments.

Slide No. 3 shows the bacilli in a section of the kidney from the case in the Sandwich Islands; and in view of this it is interesting to note the identical appearance of the bacilli, as compared with those from the case at Vienna. The latter being a native of Norway, in which country he contracted the disease, these two cases occurred in almost opposite quarters of the globe, yet the bacilli are apparently identical in both cases.

From the cover-glasses, on which I had dried the serum obtained from the leprosy nodules of the skin, presuming that the drying had not killed the spores, I inoculated eight tubes of sterilized, solid blood serum. These I placed in an incubator at a temperature of 98° F., for seven days; at the end of which time the growth made its appearance. As, however, the spores of other bacilli had evidently been present on the cover-glasses, producing various growths, fractional cultivation by several successive inoculations was necessary to entirely isolate the leprosy bacillus, which was finally done. From a pure culture thus obtained I inoculated six rabbits, inserting the bacilli through a small incision at the base of the ear, where the animal could not lick it. The rabbits were hypnotized, the hair clipped off, and the part carefully washed with a corrosive sublimate solution.

Of the six rabbits thus treated, in three the wound healed in a very short time, and, although kept under observation for a long period no sign of the animals having contracted leprosy manifested itself. A small portion of the skin and subcutaneous tissues at the site of the attempted inoculation was excised and some twenty sections cut from it, stained and examined; but none showed any trace of there having been an increase in the number of bacilli, nor any attempt at spore formation. The internal organs exhibited little, if any, change.

In the fourth rabbit there was, at the end of about three weeks, at the site of the inoculation, some sloughing and a slight secretion of pus, which latter contained a large number of the leprosy bacilli, and many of the bright, glistening spores. Cultures were made from this pus, after heating to the boiling point of water, to kill other bacteria, but not continuing it long enough to kill the spores of bacilli. Successive inoculations, on the plan of fractional cultivation, finally isolated the bacillus of leprosy, in a typical culture.

In the fifth rabbit there appeared at the end of some months a decidedly leprous condition, which was, however, almost entirely local. A small, firm nodule gradually made its appearance at the site of inoculation, and, though very small, it had most decidedly the characteristics of a leprous tubercle; which indeed microscopical examination subsequently showed it to be. This animal I then killed, and burned the carcass, not thinking it advisable to carry the experiments further; although I had all along been very careful, by effectual isolation of the inoculated rabbits, not to run any risks of infecting any person or animal.

The sixth rabbit died on the ninth day, of tetanus.

In three other rabbits I injected small portions of a pure culture into the anterior chamber of the eye; producing some opacity, and considerable growth of bacilli, but none of the characteristic tubercles, nor giant cells.

I made careful observations of the mode of growth of *Bacillus lepræ.* A cover-glass, in the center of which had been deposited a drop of sterilized blood serum inoculated with the bacilli, was placed, culture downward, on a cell slide. The latter was made by cementing a glass ring to the center of a glass slip, making, with the cover-glass, a closed cell in the bottom of which was placed a drop of water to maintain the moisture of the culture. The cover-glass was sealed in its position on the top of the glass ring with olive oil, as it is not necessary to admit air, the spores germinating freely independent of its admission. The whole, prepared in this manner, was placed on the stage, and all of that portion of the microscope below the focusing apparatus was enclosed in a box kept at a temperature of about 100° F. This arrangement afforded the means of observing everything taking place in the culture thus placed immediately beneath the cover-glass; which latter, being of the ordinary thickness, permitted of the same facility of observation as an ordinary slide preparation.

Of course the cultures are very liable in their preparation to be contaminated; but by preparing a number it is quite possible, if care be taken, to procure several in which the lepra bacillus is present.

From among a dozen I succeeded in getting a sufficient number to carefully observe the germination and growth from spore to mature bacillus. The spore would swell, then the sharp outline would fade and become transparent at a certain portion of its circumference. From this spot would appear a faint, pale projection, which would grow in length until it reached the size of a mature organism — the outlines of the original spore entirely disappearing. The new bacillus would divide into two, and these into four, and so on *ad infinitum.*

The microscopical character and general morphology of *Bacillus leprae* greatly resemble *Bacillus tuberculosis.* The giant cells in which the latter is found displaying great similarity to the large leprosy cells of Virchow, in which *Bacillus leprae* occurs. One point of difference, however, is found in the fact that while the bacilli of tubercle are not motile, some of the bacilli of leprosy are. Then another difference consists in the fact that when animals are inoculated with *Bacillus tuberculosis* it readily produces a characteristic, general and local, definitely tubercular lesion; while the bacillus of leprosy is only with difficulty inoculable in the lower animals, and in man probably requires a certain predisposing condition, just as a phthisical tendency is usually necessary for the development of consumption.

Still another point of difference is in the staining reaction, as the bacillus of leprosy takes the color more readily than does *Bacillus tuberculosis;* although the same staining processes and reactions are applicable to both.

Great similarity is also apparent between the general microscopical appearances of the nodules of leprosy and those of lupus vulgaris; both being classed as infective granulomata by the pathologist. Lupus shows the same giant cell, present in both leprosy and tuberculosis; in fact many pathologists consider lupus a local tuberculosis of the skin, as the bacillus of the latter process has been frequently, though not constantly, found in the former. But as not all of you are interested in these purely pathological questions, any discussion of them would be out of place here.